which are obtained in a medium based on POE (Figure 11a), based on POP-POE-POP at 25°C (Figure 11b) and based on POP-POE-POP at 45°C (Figure 11c).

Figure 12: Band widths for a series of separations of single-stranded DNA fragments in denaturing medium (fluorescein 50 bp ladder, Pharmacia Biotech), in media according to the invention which can be differentiated by the average number and the average length of the segments with LCST: smaller band widths correspond to a better separation.

EXAMPLE 1:

Preparation of a macromonomer with LCST of pNIPAM which is nonfunctionalized, having a molecular mass in the region of 10 000, for the preparation of a thermogelling copolymer in accordance with the invention.

1) Polymerization of NIPAM

The free-radical polymerization of NIPAM is carried out in pure water, at a temperature slightly higher than room temperature but less than the LCST of the polymer. The initiator is a redox pair in which the oxidizing agent is potassium persulphate, $K_2S_2O_8$ (KPS), and the reducing agent is aminoethanethiol, AET.HCl. The priming reaction is:

$$K_2S_2O_8 + 2Cl^-, NH_3^+-CH_2CH_2-SH \rightarrow 2KHSO_4 + 2Cl^-, HN_3^+-CH_2-CH_2-S^\bullet$$

The AET.HCl also plays the role of transfer agent, which makes it possible to control the length of the chains.

Procedure

20 g of NIPAM (0.18 mol) and 200 ml of water are introduced into a 500 ml three-necked flask surmounted by a condenser and equipped with a device for admitting

nitrogen. The mixture is then stirred and heated to 29°C by a water bath. Nitrogen bubbling is initiated. After 45 minutes, 0.42 g of AET.HCl (0.0037 mol), previously dissolved in 20 ml of water, is added, followed by 0.0018 mol of potassium persulphate (KPS) dissolved in a minimum quantity of water. The mixture is kept stirring for 3 hours. The solution is then concentrated and then freeze-dried.

To isolate the polymer, precipitation is carried out according to the following procedure:

The solid obtained is redissolved in 100 ml of methanol, the hydrochloride present is neutralized by addition of 0.0037 mol of KOH (that is 0.208 g dissolved in about 25 ml of methanol) incorporated dropwise into the solution. The salt formed, KCl, precipitates and is extracted by filtration. The filtrate thus recovered is concentrated and then poured dropwise into 4 litres of ether. The polymer precipitates and is recovered by filtration on No. 4 sintered glass. The solid is then dried under vacuum produced by a slide vane rotary vacuum pump. The mass yield is of the order of 50%.

The above protocol leads to an aminated polymer "PNIPAM-A-10", and corresponds to initiator-monomer ratios Ro=0.02 and Ao=0.01, where:

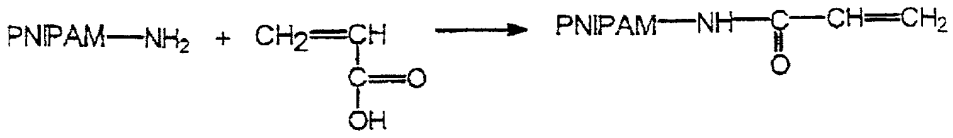
$$Ro = [R\text{-}SH]/[NIPAM] \text{ and } Ao = [KPS]/[NIPAM].$$

Various other aminated polymers were prepared according to the same protocol by varying the polymerization temperature and the ratio Ro, while maintaining a ratio Ao of 0.01. These polymers are described and defined in Table 1.

2) Modification of the aminated PNIPAM for its copolymerization with one or more segments which are soluble at the temperatures T1 and T2

The PNIPAM macromolecules synthesized have amine functional groups at the chain ends, the latter being derived from the initiator aminoethanethiol AET.HCl.

By reacting the amine functional group with acrylic acid, a vinyl double bond is attached at the end of the chain according to the following reaction scheme:



Procedure

50 ml of methylene chloride, 1.5 g of acrylic acid (0.021 mol), 9 g of PNIPAM and 4.3 g of dicyclohexylcarbodiimide (DCCP) (0.21 mol) are introduced into a 100 ml beaker.

The reaction medium is stirred for one hour. As the acrylic acid is in great excess relative to the PNIPAM (the quantity of acrylic acid is about twenty times that of the PNIPAM), it can be assumed that all the amino functional groups have been modified, which will be confirmed by the copolymerizations described in Example 2. The mixture is then filtered on No. 4 sintered glass in order to remove the dicyclohexylurea precipitate, a by-product resulting from the conversion of the DCCI.

The mixture is then concentrated to 15 ml and then poured dropwise into 200 ml of ether in order to precipitate the polymer. The mixture is filtered on No. 4 sintered glass and the solid is washed with three times 100 ml of ether and then it is dried under vacuum produced by a slide vane rotary vacuum pump overnight.